

## Bacteriological And Biochemical Studies On Pekin Duckling Infected With *Pasteurella Miltocida* With Trial For Treatment

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### ABSTRACT

Samples from cloacal swabs, liver, heart, lungs, trachea, spleen and nasal exudate were collected from 150 pekin duckling aging 1-30 days (75 apparently healthy 35 diseased and 40 freshly dead) for bacteriological examination. Out of 150 examined sample 43 (28.67 %) were positive for *Pasturalla milt.* (*P. milt.* ) distributed as 5 from apparently healthy, 13 from diseased and 25 from dead duckling. Antibiogram study of isolates revealed that florfenicol was the highest drug effective against *P. milt.* .

A total of 160, one day old pekin duckling (80 healthy & 80 experimentally infected with *P milt.*) were divided into 4 equal groups ( 40 birds each), the 1<sup>st</sup> group consisted of healthy duckling (control), the 2<sup>nd</sup> group included healthy duckling treated with florfenicol (30mg/kg bwt.) in drinking water for 5 days, the 3<sup>rd</sup> group included infected non treated and the 4<sup>th</sup> group included infected duckling treated with florfenicol (same dose, period and rout of administration). In the four groups the hemato-biochemical changes were studied.

The results revealed that healthy pekin duckling treated with florfenicol displayed significant rise in body weight, leukocytic count, lymphocytes, significant decrease in heterophils and insignificant increase in monocytes, eosinophils, basophils, total proteins, albumin, globulins, A/G ratio, AST, ALT, ALP, uric acid and creatinine.

Pasteurellosis in duckling induce anorexia depression, ruffled feather, coughing, diarrhea, mortality rate 30%, rise respiratory rate, monocytes, total,  $\beta$ ,  $\gamma$  globulin, AST, ALP, uric acid, creatinine levels and significant decrease in weight gain, heterophils, albumin,  $\alpha$  globulin, A/G ratio, insignificant decrease in leukocyte, lymphocyt, esinophils, total protein and insignificant rise ALT

Florofenicol residue in examined liver and kidney in both healthy and diseased duckling treated with florofenicol were high at 1<sup>st</sup> day post treatment, very low at 6<sup>th</sup> day and completely disappeared from examined samples at 9<sup>th</sup> day post treatment The highest levels of florofenicol residues were recorded in the kidneys then liver.

Duckling suffering from pasteurlosis and treated with florfenicol showed no clinical signs, decrease mortality rate to 5%, reduced re-isolated *P. milt* and Improve hemato-biochemical parameters.

It could be conclude that florfenicol is effective against *P. milt* in duckling

### INTRODUCTION

Ducks are relatively resistant to some diseases (1). In duck farms in Egypt, inadequate management, diagnosis, control and prevention of various infectious diseases play

a vital role in high morbidity and mortality (2). Pasteurellosis is a contagious disease in ducks caused by *p. milt* (3) which is associated with poor sanitation (4). Acute form occurs as septicemia of sudden onset with high mortality

(5). While chronic form results in localization of infection in wattles, respiratory passages and joints (6). Symptoms of pasterulosis include anorexia, mucous discharge from mouth, diarrhea and labored breathing (7).

Florfenicol is a novel broad spectrum bacteriostatic antibiotic belonging to family includes also thiamphenicol and chloramphenicol (8). It has fluorine atom instead of hydroxyl group located at C-3 in the structure of chloramphenicol and thiamphenicol (9). Florfenicol inhibits protein synthesis (10), it has greater activity than chloramphenicol and thiamphenicol against *Pasteurella* (11).

The aim of this study was to evaluate effects of pasteurellosis on immunological and biochemical parameters in duckling with trial of treatment.

## MATERIAL AND METHODS

### Isolation and identification

A total of 150 samples (35 diseased, 40 freshly dead, 75 healthy pekin duckling) 1-30 days old were obtained from different private farms at Sharkia Province. All samples were collected aseptically from cloacal swabs, liver, heart, lungs, trachea, spleen and nasal exudate and inoculated into nutrient broth aerobically at 37°C over night, subculturing on nutrient agar and MacConkey agar plates was performed for 24h at 37°C, suspected colonies were identified (12). Pathogenicity and virulence of isolated *P. milt* to mice were determined (13).

### Antibiotic sensitivity test

Susceptibility of *P. milt* to different chemotherapeutic agents was tested by disc diffusion method (14).

### Drugs

Florfenicol (Aviflor, 100 mg/ml) water soluble formulation for oral use was supplied by Avico (Jordan).

### Experimental duckling

A total of 160, one day old pekin duckling were reared under hygienic conditions, Fed on balanced commercial starter ration free from antibacterial agent and given water ad-libitum,

### *Pasterulla miltocida* infection

On day 30 of age 80 duckling were I/M inoculated with 0.2 ml/bird of 48 hr broth culture of *P. milt.* containing ( $3 \times 10^8$  CFU) viable organism (15).

### Experimental design

Duckling were divided into 4 equal groups (40 ducks each), 1<sup>st</sup> group included non-infected non-treated duckling (control group), 2<sup>nd</sup> group included non-infected duckling treated with florfenicol (30 mg/kg bwt) in drinking water for 5 successive day. The 3<sup>rd</sup> group included infected non treated duckling and the 4<sup>th</sup> group included infected duckling treated with florfenicol (same dose, period and route of administration). Treatment started at age of 32 day.

### Body weight

From each group 5 ducklings were weighted individually at the start of the experiment and at the 1<sup>st</sup> day post treatment and consumed diets were recorded the calculation of weight gain and feed conversion rate.

### Sampling

At 1<sup>st</sup>, 7<sup>th</sup> & 15<sup>th</sup> days post treatment two blood samples from all duckling were taken, the 1<sup>st</sup> sample was taken on tube contain EDTA to estimate leukogram (16). The 2<sup>nd</sup> one was taken to obtain clear serum to estimating of the total protein (17). Protein fraction was performed using cellulose acetate electrophoresis (18). (AST-ALT) (19) ALP (20) uric acid (21) creatinine (22).

### Re-isolation of *Pasterulla milt*

Samples were taken aseptically from cloacal, nasal exudates and internal organs from all groups post treatment then inoculated into nutrient broth aerobically at 37°C over

night followed by subculturing on nutrient agar and MacConkey agar plates for 24h at 37°C, suspected colonies were identified (12). Pathogenicity and virulence of isolated *P. milt* to mice were determined (13).

#### Drug residue

Five duckling from both healthy treated and infected treated ducklings were slaughtered at 2<sup>nd</sup> and 3<sup>rd</sup> days during treatment and at 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup> & 9<sup>th</sup> days post treatment. Levels of florfenicol residues in liver and kidneys were determined by microbiological assay technique (23) using *Baceillus subtilis* as test organism

**Statistical Analysis:** Obtained data were statistically analyzed using T test (24).

## RESULTS AND DISCUSSION

Bacteriological examination revealed that 43 samples (28.67%) out of 150 collected samples to be positive for *P. milt* (5 from healthy, 13 from diseased and 25 from freshly dead duckling). *P. milt* was isolated from diseased Muscovy ducks in percentage of 25% (25). Pasteurellosis in duckling induce clinical sign as depression, anorexia, ruffled feathers, increase respiratory rate, sneezing, coughing, diarrhea, mortality rate 30% and decrease body weight. Typical signs were previously recorded in duckling (26).

The obtained results revealed that, use florfenicol in healthy ducks displayed a significant increase in body weight gain and decrease feed conversion rate in comparasion to healthy duckling, meanwhile, pasteurellosis induce significant decrease in body weight gain and feed conversion rate in comparasion to healthy duckling. The antimicrobials drugs induce rise in growth rate through inhibiting pathogenic organisms (27). Pasteurellosis in duckling induce significant decrease in body weight gain and increase in feed conversion rate (28).

Antibiogram revealed that psterulla multocida was sensitive to florfenicol and ceftiofur sodium followed by gentamycin, spectinomycin and not sensitive to colistin. Same reports showed that *P. milt* were very sensitive to florfenicol (25). Other report that *P. milt* were resistant to colistin (29).

Florfenicol induce significant leukocytosis, lymphocytosis, significant decrease in heterophils, insignificant rise in monocytes, eosinophils and basophils. Pasteurellosis in duckling showed insignificant decrease in leukocytic count, lymphocyt, esinophil, significant decrease in heterophils and monocytosis. Same results were previously reported in healthy rats received florfenicol for 6 day (30). Our result agreed with those obtained in broiler chicken infected with *P. milt* (31). Pasteurellosis in duckling induce insignificant reduction in lymphocyt beside significant decrease in heterophils and monocytosis (26).

In the current work, florfenicol displayed insignificant increase in total proteins, albumin globulins and A/G ratio. Pasteurellosis in duckling show insignificant hypoproteinemia, significant rise in total,  $\beta$ ,  $\gamma$  globulin, significant hypoalbum- inemia,  $\alpha$  globulin and A/G ratio. Same results were recorded in healthy broiler chicken treated with florfenicol (32-33). Changes in protein profile in duckling suffering from pasteurellosis agreed with (34) in duckling and (35) in chicken. Reduction in albumin in duckling infected with *P. milt* may be due to effect of bacteria and its toxin in liver (sole of albumin synthesis) (36). Rise in  $\beta$  and  $\gamma$  globulin indicate activation of immune system due to infection (37). Reduction in globulin was recorded in infected duckling indicating immune defense mechanism against infection and enhanced immunoglobulin synthesis (38).

Healthy duckling treated with florfenicol revealed insignificant increase in AST, ALT ALP, uric acid and creatinine. Pasteurellosis in duckling induce significant rise in AST, ALP, uric acid and creatinine levels beside insignificant rise ALT. Same results were obtained in healthy chickens treated with

florfenicol (39). Florfenicol had no adverse effects on liver and kidney functions (40). Same changes in liver enzymes, uric acid and creatinine in duckling suffering from pasteurellosis were previously recorded (41). Rise in liver enzyme activities may be due to liver damage by infectious agent and its toxins (42). Elevation in uric acid and creatinine could be due to effect *P. milt* or its toxin on kidney (43).

The obtained results revealed that florfenicol residues in liver and kidney in both healthy and diseased duckling treated with florfenicol were high at 1<sup>st</sup> day of clearance period and completely disappeared at 9<sup>th</sup> days of clearance period. High residue of florfenicol was found in kidney followed by liver. Florfenicol residues in liver and kidney disappeared at 8 day of clearance period

(44,45). Florfenicol residues in kidney more than in liver (46,47).

Our findings revealed that duckling infected with *P. milt*. and treated with florfenicol showed disappearance of clinical signs, reduced mortality rate (5%), ameliorate the adverse effects and return leukogram and biochemical parameters to normal levels. This finding was previously recorded (26) which showed that duck pasteurellosis treated with florfenicol induce improvement of clinical signs and hemato- biochemical parameters. This improvement may be due to antimicrobial effect of florfenicol (27).

From the obtained results in the current study, it could be conclude that florfenicol is effective against *P. milt* in duckling

**Table 1. Incidence of the isolated *P. milt* from ducks**

Source of sample	Total No. of sample	+ve samples		-ve samples	
		No.	%	No.	%
Apparently Healthy duckling	75	5	6.67	70	93.33
Disased duckling	35	13	37.14	22	62.86
Freshly dead duckling	40	25	62.50	15	37.50
Total	150	43	28.67	107	71.33

**Table 2. Incidence of the isolated *P. milt* from internal organ in ducks**

Source of sample	Healthy			Disased			dead		
	Total sample	+ve sample		Total sample	+ve sample		Total sample	+ve sample	
		No	%		No	%		No	%
Tracheal swab	35	3	8.57	5	2	40	8	4	50
Cloacal swabs	40	2	5	3	1	33.33	6	3	50
Liver	-	-	-	9	2	22.22	7	4	57.14
Heart	-	-	-	6	2	33.33	3	3	100
Lung	-	-	-	3	2	66.67	9	6	66.67
spleen	-	-	-	5	1	20	7	5	71.43
nasal exudates	-	-	-	4	1	25	-	-	-
Total	75	5	6.67	35	13	37.14	40	25	62.5

**Table 3. Effect of *P. milt* on mortality rate and reisolated *p. Maltocida* of duckling**

Group	Parameters	Total No	Clinical Signs	Mortality rate		Reisolation	
				No	%	No	%
Healthy non treated (Control)		40	00	00	00	00	00
Healthy Florofenicol Treated		40	00	00	00	00	00
Diseased non treated		40	38	12	30	40/40	100
Diseased treated		40	2	3	5	3/40	7.5

**Table 4. In vitro susceptibility *p. milt* to some commonly used antibiotics**

Antimicrobial agent	Disk polancy	Inhibition zone diameters(mm)	Sensitive
Florphenicol	30ug	25	+++
Ceftiofur sodium	30ug	25	+++
Sulfaquanoxaline	30ug	24	+++
Gentamycin	10ug	23	+++
Spectinomycin	10ug	22	++
Colistine	30ug	16	R

+++ = high sensitive      ++ = sensitive      R = Resistance

**Table 5. Effect of *p. milt* and florofenicol on body weight gain (gm) and feed conversion rate in duckling (n=5)**

Parameter	Control group	Healthy treated	Diseased Non treated	Diseased treated
Weight at 30th day of age	410.32±1.49	418.58±1.37	405.08±1.40	415.41±1.86
Body weight at 1st day PT	480.49±5.38	502.04±9.80*	464.22±3.50*	489.55±15.94
Weight gain	69.45± 1.98	83.46±3.97*	59.14± 2.13*	74.14± 1.85
Feed consumption	238.59	243.39	217.43	228.53
Feed consumption rate	3.45	2.92	3.68	3.08

\*Significant at  $P \leq 0.05$ 

PT= post treatment

Table 6. Effect of *P. milt* and florofenicol on leukogram in duckling (n=5)

Parameter	Control group	Healthy florofenicol treated	Non Treated	Diseased		
				Day post treatment		
				1 <sup>st</sup>	7 <sup>th</sup>	15 <sup>th</sup>
TLC( $\times 10^3$ )	15.87 $\pm$ 0.50	17.31 $\pm$ 0.27*	14.90 $\pm$ 0.15*	15.06 $\pm$ 0.92	15.63 $\pm$ 0.99	15.75 $\pm$ 0.65
Heterophils	4.12 $\pm$ 0.85	2.15 $\pm$ 0.19*	2.59 $\pm$ 0.37*	3.07 $\pm$ 0.40*	3.84 $\pm$ 0.63	4.08 $\pm$ 0.49
Lymphocytes	8.09 $\pm$ 0.64	10.90 $\pm$ 0.98*	7.40 $\pm$ 0.89	7.51 $\pm$ 0.78	7.77 $\pm$ 0.56	8.02 $\pm$ 0.60
Eosinophils	1.34 $\pm$ 0.35	1.57 $\pm$ 0.41	1.07 $\pm$ 0.55	1.10 $\pm$ 0.70	1.25 $\pm$ 0.69	1.30 $\pm$ 0.50
Basophils	1.17 $\pm$ 0.22	1.33 $\pm$ 0.35	1.16 $\pm$ 0.32	1.18 $\pm$ 0.62	1.18 $\pm$ 0.71	1.15 $\pm$ 0.62
Monocyte	1.15 $\pm$ 0.30	1.36 $\pm$ 0.34	2.68 $\pm$ 0.57*	2.20 $\pm$ 0.61*	1.59 $\pm$ 0.54	1.20 $\pm$ 0.93

\*Significant at  $P \leq 0.05$ Table 7. Effect of *p. milt* and florfenicol on protein profile in duckling (n=5)

Parameter	Control group	Healthy florofenicol treated	Non Treated	Diseased		
				Day post treatment		
				1 <sup>st</sup>	7 <sup>th</sup>	15 <sup>th</sup>
T. protein (g/dl)	6.09 $\pm$ 0.10	6.87 $\pm$ 0.14	5.95 $\pm$ 0.18	6.03 $\pm$ 0.22	6.08 $\pm$ 0.15	6.13 $\pm$ 0.19
Albumin (g/dl)	3.83 $\pm$ 0.42	4.35 $\pm$ 0.60	3.13 $\pm$ 0.28*	3.41 $\pm$ 0.16*	3.60 $\pm$ 0.13	3.79 $\pm$ 0.15
Globulin (g/dl)	$\alpha$	0.70 $\pm$ 0.19	0.77 $\pm$ 0.13	0.49 $\pm$ 0.10*	0.54 $\pm$ 0.08*	0.68 $\pm$ 0.17
	$\beta$	0.83 $\pm$ 0.09	0.95 $\pm$ 0.16	1.25 $\pm$ 0.12*	1.20 $\pm$ 0.07*	1.00 $\pm$ 0.12
	$\gamma$	0.73 $\pm$ 0.10	0.80 $\pm$ 0.18	1.08 $\pm$ 0.11*	0.88 $\pm$ 0.09*	0.80 $\pm$ 0.14
Total	2.26 $\pm$ 0.37	2.52 $\pm$ 0.91	2.82 $\pm$ 0.14*	2.62 $\pm$ 0.19*	2.48 $\pm$ 0.17	2.34 $\pm$ 0.18
A/G Ratio	1.69 $\pm$ 0.15	1.72 $\pm$ 0.42	1.12 $\pm$ 0.20*	1.30 $\pm$ 0.19*	1.45 $\pm$ 0.25	1.62 $\pm$ 0.33

\*Significant at  $P \leq 0.05$ Table 8. Effect of *p milt* and florfenicol on liver enzymes and kidney function in duckling (n=5)

Parameter	Control group	Healthy florofenicol treated	Non Treated	Diseased		
				Day post treatment		
				1 <sup>st</sup>	7 <sup>th</sup>	15 <sup>th</sup>
AST( $\text{U/L}$ )	30.28 $\pm$ 1.50	32.04 $\pm$ 1.78	36.04 $\pm$ 1.41*	34.78 $\pm$ 1.17*	33.20 $\pm$ 1.44	31.08 $\pm$ 1.48
ALT( $\text{U/L}$ )	24.05 $\pm$ 1.42	25.34 $\pm$ 1.89	29.13 $\pm$ 1.89	27.85 $\pm$ 1.47	26.30 $\pm$ 1.60	24.97 $\pm$ 1.93
ALP (I.U/ml)	17.09 $\pm$ 1.12	19.21 $\pm$ 1.96	21.87 $\pm$ 1.04*	21.03 $\pm$ 1.10*	19.14 $\pm$ 1.79	18.21 $\pm$ 1.43
Urea(mg/dL)	4.92 $\pm$ 1.22	5.58 $\pm$ 1.60	7.88 $\pm$ 1.10*	7.61 $\pm$ 1.03*	6.05 $\pm$ 1.02*	5.12 $\pm$ 1.40
Creatinine(mg/dL)	1.14 $\pm$ 0.11	1.25 $\pm$ 0.21	1.69 $\pm$ 0.17*	1.54 $\pm$ 0.09*	1.34 $\pm$ 0.13	1.20 $\pm$ 0.15

\*Significant at  $P \leq 0.05$

Table 9. Florfenicol residues ( $\mu\text{g/gm}$ ) in duckling liver and kidney

	Clearance period								
	Healthy duckling with florfenicol (days)				Diseased	duckling with florfenicol (days)			
	1 <sup>st</sup>	3 <sup>rd</sup>	6 <sup>th</sup>	9 <sup>th</sup>		1 <sup>st</sup>	3 <sup>rd</sup>	6 <sup>th</sup>	9 <sup>th</sup>
Kidney	6.72 $\pm$ 0.21	2.15 $\pm$ 0.11	0.31 $\pm$ 0.18	00	6.41 $\pm$ 0.14	2.07 $\pm$ 0.18	0.19 $\pm$ 0.07	00	
Liver	4.07 $\pm$ 0.19	1.17 $\pm$ 0.2	0.25 $\pm$ 0.15	00	4.12 $\pm$ 0.2	1.3 $\pm$ 0.10	0.10 $\pm$ 0.06	00	

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### الملخص العربي

دراسات بكتريولوجية بيوكيميائية على البط البيكيني المصاب معمليا  
بالباستريلا مع محاوله العلاج

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أقسام (الدواجن<sup>١</sup> الكيمياء<sup>٢-٣</sup> و<sup>٤</sup>)، معهد بحوث صحة الحيوان (فرع الزقازيق<sup>١-٣</sup>) كفر الشيخ<sup>٣</sup> واسماعيليه<sup>٤</sup>

تم تجميع عدد ١٥٠ عينة من الأعضاء الداخلية لبط بيكيني عمر ١ حتى شهر (٧٥ بطه سليمة ظاهريا، ٣٥ بطه يظهر عليها أعراض متمثلة في الإسهال والكساح و ٤٠ بطه ناقفه حديثا) للفحص البكتريولوجي. وبعد الفحص البكتريولوجي وجد ميكروب الباستريلا مالتوسيدا في عدد ٤٣ عينة (٢٨,٦٧%) من ١٥٠ عينة تم فحصها موزعه كالاتى (٥ بطه سليمة، ١٣ بطه مريضه و ٢٥ بطه ناقفه. وبعمل اختبار الحساسية لتلك المعزولات وجد أن الفلورفينيكول أكثر المضادات الحيوية المستخدمة تأثيرا على البستريلا مالتوسيدا المعزولة عن باقي المضادات الحيوية المستخدمة.

في هذه الدراسة تم استخدام عدد ١٦٠ من البط البيكيني عمر يوم واحد (٨٠ بطه سليمة و ٨٠ بطه مصابه اصطناعية بالباستريلا مالتوسيدا. قسم البط إلى أربع مجموعات متساوية. المجموعة الأولى بط سليم

ظاهريا واكلينكيا ولم يعالج (مجموعة ضابطة) والمجموعة الثانية بط سليم ظاهريا واكلينكيا وتم علاجه باستخدام الفلوروفينيكول بجرعة ٣٠مجم/كجم من وزن الجسم لمدة ٥ أيام متتالية, المجموعه الثالثة بط مصاب اصابه اصطناعية بالباستريلا مالتوسيدا ولم يتم علاجه إما المجموعه الرابعة بط مصاب اصابه اصطناعية بالباستريلا مالتوسيدا ويتم علاجه باستخدام الفلوروفينيكول بنفس الجرعة والمدته السابقه. يتم دراسة تأثير الباستريلا على نسبة الوفيات ووزن الجسم. يتم اخذ عينات دم من البط في كل المجموعات وذلك لقياس بعض الوظائف المناعية والبيوكيميائية. يتم ذبح عدد ٥ بطه عند فترات مختلفة إثناء وبعد نهاية العلاج ويتم اخذ عينات من الكبد والكلى لتعيين بقايا الفلوروفينيكول.

وأظهرت النتائج أن البط السليم والمعالج بالجرعة العلاجية من الفلوروفينيكول أدى إلى حدوث زيادة معنوية في وزن الجسم, العدد الكلى لكرات الدم البيضاء, الخلايا الليمفاوية ونقص معنوي في خلايا الهيتروفيل بجانب زياده غير معنويه في معدل التحويل الغذائي الخلايا الملتهمه الكبيرة, الخلايا القاعدية, الخلايا الحامضيه, البروتين الكلى, الزلال, النسبه بين الزلال والجلوبيولين, حمض اليوريك والكرياتينين AST, ALT, ALP. حمض اليوريك والكرياتينين

البط المصاب اصابه اصطناعية بالباستريلا مالتوسيدا ظهرت عليها أعراض مرضيه تتمثل في حدوث أصوات غير طبيعية والامتناع عن الأكل والإسهالات بجميع الألوان وارتفاع نسبة النافق ٣٠% و وجود زياده معنويه في عدد الخلايا الملتهمه الكبيرة الجلوبيولين الكلى والبيتا والجاما جلوبيولين, AST, ALP, حمض اليوريك والكرياتينين بجانب نقص معنوي وزن الجسم, خلايا الهيتروفيل, الزلال, الفا جلوبيولين, النسبه بين الزلال والجلوبيولين ونقص غير معنوي في معدل التحويل الغذائي العدد الكلى لكرات الدم البيضاء, الخلايا الليمفاويه, الخلايا الحامضيه, البروتين الكلى وزيادة غير معنويه في ALT

وقد دلت نتائج الدراسة على أن الفلوروفينيكول له بقايا في الأنسجة اثنا وبعد العلاج وكان أعلى منسوب لبقايا الفلوروفينيكول في الكلى يلها الكبد, ولكن الفلوروفينيكول اختفي من الأنسجة بعد مرور ٩ يوم من الحقن

من كل ما سبق نلاحظ أن استخدام الفلوروفينيكول بالجرعة العلاجية لة تأثير فعال في علاج الإصابة بالباستريلا وأدى إلى اختفاء الأعراض الظاهرية وأدى إلي عودة هذه الوظائف إلي المستوى الطبيعي.